# STUDY OF THERAPEUTIC ROLE OF PROBIOTICS ON DIABETES MELLITUS AND ASSOCIATED METABOLIC DISORDERS

A Synopsis Submitted in

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# SYNOPSIS

## 1. TITLE OF THE PROJECT:

**STUDY OF THERAPEUTIC ROLE OF PROBIOTICS ON DIABETES MELLITUS AND ASSOCIATED METABOLIC DISORDERS.**

## 2. INTRODUCTION:

Probiotics are described as live microbial feed supplements that beneficially affect the host animal by improving its intestinal microbial balance. Several different types of products which are formulated as drugs or other dietary supplements, actually originate from live microbes called probiotics. Various micro-organisms are considered potent probiotics based on their biological and clinical aspects. The majorities of these organisms belong to the LAB (Lactic Acid Bacteria) group. The LAB predominantly includes organisms which belong to the genera of *Lactobacillus* and *Bifidobacterium* but from varied species and strains which altogether is considered as potential probiotic (Kleerebezem and Vaughan, 2009).

More than 500 million patients are affected by diabetes worldwide. Diabetes patients have impairment in healing because of imbalance of the inflammatory response, prolonged aggregation of inflammatory cells and excessive formation of inflammatory cytokines. These factors result in reduced wound resistance and thereby cause increase in hospitalization time and mortality rate. Probiotic intervention has been seen to improve insulin sensitivity, reduce fasting blood glucose, boost up antioxidant activity and inflammatory status in type 2 diabetes patients. Some studies have provided evidence that probiotics can prevent and sometimes even improve gestational DM (Kesika *et al.,* 2019). Different meta-analysis studies and clinical trials have shown that treatment with probiotics successfully reduced blood pressure, lipid profile and other risk factors associated with T2D (Kasińska and Drzewoski, 2015).

Metabolic syndrome is a group of medical conditions that elevate the risk factor for cardiovascular diseases and other health complications such as stroke and type 2 diabetes. It is a condition of low-grade chronic inflammation which includes serious diseases such as hyperglycemia, hyperlipidemia, hypertension and obesity (de Toro-Martín *et al.,* 2017). Bioactive dietary ingredients, like probiotics, prevent or slow the progression of Metabolic disorders, Diabetes Mellitus, Obesity and Cardiovascular health disorders by improving the most significant risk factors, such as gut dysbiosis.

Some of the most significant functions of probiotics in maintaining proper human health are as follows:

- They thrive and replicate on our intestinal surfaces, create different antimicrobial substances and forms a stable system that protects us from the invasion of pathogens (Mills *et al.,* 2019)
- They play a key role in the metabolism and proper functioning of our digestive system (Rothschild *et al.,* 2018)
- They modify insulin resistance and influence insulin secretion (Kelly *et al.,* 2015)
- They impact brain-gut communication and promote proper neurological functioning in the host (Zheng *et al.,* 2019)

The relationship between the wound healing process after injury and the interaction with the skin microbiota is already well established. Recent studies suggest that, changes in the intestinal microbiota may positively affect the wound healing process by producing antimicrobial molecules, and regulating the inflammatory response. Thus, modulating the intestinal microflora might be an important strategy for improving the skin healing process. Probiotics and synbiotics can be used as tools for modulation of the intestinal microbiota.

Till date, there have been many studies showing the link between diabetes and delay in wound healing. On the other hand, various studies have also established the use of probiotics in the treatment of different kinds of wounds in patients. However, not much research work has been done to understand whether there is any direct link between probiotic application and its beneficiary effect in healing the wounds caused specifically due to diabetes and associated diseases. The research in this domain is still at a very nascent stage regarding the exact mechanism of action of probiotics on wound healing and other immunological parameters, particularly in case of patients suffering from diabetes mellitus and metabolic syndrome. We have been able to identify a research gap in this area of interest and think that further research is required.

## 3. RESEARCH OBJECTIVES:

- Isolation and selection of probiotic bacteria from different indigenous sources.
- Induction of diabetes in rodent model followed by induction of various types of wounds in the diseased rodent.
- Application of probiotics and studying the effects of probiotics on wound healing in diseased rodent model.
- Immunomodulatory study of the probiotics in diseased rodent.

## 4. REVIEW OF LITERATURE:

The word 'probiotic' is derived from the Greek term 'pro bios' which means 'for life'. This term was coined by German scientist Werner Kollath in 1953. He defined probiotics as "active substances that are essential for healthy development of life." In 1965, scientists Lilly and Stillwell were the first to narrate that probiotics are secreted from living organisms themselves, which in turn, stimulate the growth and development of other microorganisms. The most modern and widely accepted definition of probiotics describes them as live microorganisms which on administration in adequate amounts; provide certain health benefits to the host. Probiotics are proposed to be a potent therapeutic against different health disorders due to their antimicrobial activity, antioxidant activity, capability to regulate the immune system and modulate the intestinal microbiome. The beneficial health effects of probiotics are broadly categorized into three sections, namely, maintenance of the gut microbial ecosystem, metabolic effect on the intestine and immune-modulation (Bermudez-Brito *et al.,* 2012). Their daily consumption in adequate quantities also helps to restore normal gut microbiota, resulting in improved insulin signalling and immune responses, maintenance of lipid homeostasis and decrease in low-grade chronic inflammation (Sun *et al.,* 2020).

Studies have shown that probiotic bacteria express antioxidant abilities both *in vivo* and *in vitro*, by scavenging the free radicals (Lin and Yen, 1999). In Type 2 Diabetes patients, the oxidative stress can be significantly reduced by multispecies probiotics (Asemi *et al.,* 2013). Probiotics can produce several metabolites having antioxidant activity, such as butyrate, folate and glutathione. Treatment with probiotics can also regulate the levels of antioxidant metabolites of the host. It has been seen that the daily consumption of yoghurt containing *Lactobacillus acidophilus* improves the mean levels of vitamin B12 and folate in Type 2 Diabetes patients. Vitamin B12 and folate, in turn, are able to rescue cells from oxidative stress.

Probiotics have also been seen to express antimicrobial activity against invasion of pathogens in our skin and are used to treat skin conditions like surgical site skin infections, skin lesions and foot ulcers (Valdéz *et al.*, 2009). The anti-microbial property of probiotics is mainly due to their capability to produce certain compounds called bacteriocins, which are active against both gram positive and gram negative bacteria. The immense changes and influence that probiotic bacteria have on the immune system has led to the interest of researchers to exploit this trait as future treatment for diseases and ailments. Probiotic bacteria are popular in promoting the host's shielding mechanisms. Probiotics can be used as cytokine modulators. The capacity of probiotics to activate or decrease cytokine production has been established in several investigations. Modulation of cytokine has a direct impact on the immune function of the host cell and hence use of probiotics as cytokine modulators may be useful in prophylaxis and treatment of immune related conditions. The entire action of the host immune system is mediated through macrophage activation, cytokine augmentation, NK cell activation, and antibody formation (Ouwehand *et al.*, 2002).

Recent studies have showed that the host microbiota plays an influential role in the management of diabetes mellitus, especially in Type 2 Diabetes. Dysbiosis of gut microbiota is strongly linked to T2D and it has been observed that the gut microbiota of T2D patients comprises of increased number of opportunistic pathogens and decreased number of beneficial microbes, specifically butyrate producing bacteria. Probiotic supplemented T2D patients have shown improvement of gut microbial composition. The oral supplementation of probiotics has been suggested to be an effective strategy to alter gut microbiota composition in diabetic population (Festi *et al.,* 2014). The oral supplementation of probiotics has been observed to lessen the risk of diabetes by suppressing the rise of blood cholesterol and having anti-obesity effects (Barengolts, 2016). Treatment with probiotics successfully lowered total cholesterol, blood pressure, low density lipoproteins, triglycerides and other risk factors linked with T2D, according to many meta-analysis studies and clinical trials (Kasińska and Drzewoski, 2015). Probiotics can also reduce cardiovascular risk by controlling dyslipidemia and hypertension in patients with T2D (Mazloom *et al.,* 2013).

The skin is an important organ that acts as the first line of defence against external infection by serving as a physical barrier to prevent invasion of pathogens. Skin can be damaged as a result of surgery, illness, and burns, or as a result of underlying diseases like diabetes. The most effective strategy to manage skin wounds is to prevent infection and promote healing (Mihai *et al.,* 2018). Research has shown that here is a relationship between gut microbiota, immune system, and skin health and healing (Al-Ghazzewi and Tester, 2014). Multiple studies describe that probiotic supplementation therapy using *Lactobacillus plantarum, Lactobacillus casei* and *Lactobacillus acidophilus* have improved wound healing by increasing tissue repair and reducing the bacterial load in

wounded rodent models (Huseini *et al.,* 2012). Probiotics can help in skin healing by boosting the production of immune cells and exhibiting antagonistic activity by competitively excluding the pathogens (Fijan *et al.,* 2019). In case, there is a disruption in the natural balance of the skin microbiome, probiotics can confer a positive effect on skin healing by producing bioactive metabolites that inhibit pathogen growth. Probiotics are also capable of coaggregating with pathogens and facilitating their removal or displacement from skin (Lukic *et al.,* 2017). Antimicrobial resistance is an issue of great concern nowadays because it poses a serious threat to humans. Therefore, alternatives to topical skin antibiotics are gathering great interest. The OECD has stated that probiotics are a potential alternative treatment strategy to topical antibiotics (Kadam *et al.,* 2019). Studies have established that the topical application of probiotics on skin abrasions, ulcers and burns causes sufficient reduction of pathogen load (Sikorsa *et al.,* 2013).

## 5. YEAR-WISE WORK PLAN:

| TIME | WORK TO BE DONE |
|---|---|
| YEAR-1 | Choice of appropriate sources and collection of sources |
| | Isolation of probiotics from selected sources |
| | Biochemical characterisation of isolated probiotics |
| | Molecular characterisation of isolated probiotics |
| | Arrangement of rodents and their acclimatization |
| | Induction of Diabetes Mellitus and Metabolic disorders by treatment with specified chemicals and by dietary interventions |
| YEAR-2 | Induction of different kinds of wounds in the diseased rodents using established physical and chemical methods |
| | Treatment with different combinations and dosages of prebiotics, probiotics and synbiotics |
| | Study of organ physiology and assessment of wound contraction rate of rodent models |
| YEAR-3 | Immunomodulatory assays to determine the effect of probiotic, prebiotic and synbiotic supplementation on diseased and wounded rodent models |
| | Analysis and interpretation of the data collected |
| | Thesis writing and final editing |
| | Thesis submission |

## 6. METHODOLOGY :

- **Isolation of probiotic organisms from different sources (fermented rice water, buffalo milk, goat milk, human breast milk):**
  The isolation of probiotic organisms from different sources is done by performing agar plating and pure culture method.

- **Characterization of pure culture isolates:**
  The pure culture isolates are then subjected to different tests for the confirmation of their role as probiotics and selected for further study accordingly.

**I) Phenotypic screening:**

a) Gram staining
b) Endospore staining
c) Urease test
d) Oxidase test
e) Catalase test
f) Amylase test
g) Carbohydrate utilization test (glucose, lactose, galactose, maltose, erythrose, sorbitol)
h) Anti-microbial activity (Agar cup diffusion and MIC determination)
i) Anti-oxidant activity (DPPH Assay)
j) Bile salt tolerance test
k) Determination of optimum pH and optimum temperature.

**II) Genotypic screening**:

a) Genomic DNA isolation
b) Purification of isolated DNA
c) PCR of purified DNA
d) 16s rRNA sequencing
e) Accession number generation

- **Animal Experimentation (Rodent Model) and disease induction:**

Animal chosen for the experiment is rodent. 3 groups of rodents are taken-

**Group A:**

Rodents used as control will be neither induced with any disease nor introduced to any treatment.

**Group B:**

Disease will be induced to this set of rodents via various chemicals (alloxan monohydrate) and different types of wounds would be inflicted by surgical procedure.

**Group C:**

This set of rodents will be initially induced with disease and wounds, but finally treated with different dosages and combinations of -

i) Selected probiotic strains
ii) Selected probiotics and various prebiotics (inulin, oligosaccharides)

- **Histological Assay:**

Histological assay will be performed on tissue samples from wounded diabetic rodent model. Histological study helps to determine the various parameters of wound healing and inflammation. It is done to all the three sets of rodents for further comparative study.

- **Wound contraction assessment:**

We will macroscopically evaluate the wounds by taking digital photographs at specific intervals of time. The wound contraction rate can be analysed by measuring the wound area with the help of special software (Image-Pro Plus 4.5).

- **Immunomodulatory study:**

We will take serum samples from all three sets of rodent model and comparatively study the expression of TNF-α, IFN-γ, IL-1, IL-4, IL-6, IL-10, IL-12 and IgA by ELISA to understand the immunomodulatory aspects of probiotic and synbiotic supplementation therapy on diseased and wound inflicted mice.

## 4. SIGNIFICANCE OF THE STUDY:

- Identification of probiotics present in different sources like fermented rice water, buffalo milk, goat milk, human breast milk.
- Identification of probiotic strains that can be used in treatment of diabetes and metabolic syndrome.
- Establishment of antimicrobial activity of probiotics to fasten up wound healing in diabetic and metabolic syndrome patients.
- Establishment of antioxidant activity of probiotics to scavenge reactive oxygen species induced by diabetes and different metabolic disorders.
- Establishment of efficient probiotic and synbiotic mixture for pharmaceutical uses.

## 5. EXPECTED OUTCOME AND APPLICATIONS OF THE RESEARCH:

The main expected outcome of our study is the establishment of a direct relationship between the application of probiotics, faster wound healing and better immunological response in diabetic and metabolic syndrome patients. If we can somehow relate these factors, we would be able to find a mechanism to boost the immunological aspects of wound healing by consuming certain personalized food products. This would give us a further insight into the concept of Nutrigenetics. Such a relation would facilitate a less expensive treatment strategy for wounds caused due to diabetes. Also, it would have lesser side effects. If soldiers, sportsmen, factory workers and other people who are prone to injuries are patients of diabetes or metabolic syndrome, and they get injured someway, they would experience delay in wound healing. This can lead to septicaemia, thus increasing the necessity for amputation. Our study is targeted to verify if we can somewhat fasten up the wound healing process and boost the immune response in such patients by probiotic supplementation.

# REFERENCES


1. Al-Ghazzewi F.H., & Tester R.F. (2014). Impact of prebiotics and probiotics on skin health. *Benef Microbes,* 5(2), 99–107.
2. Asemi, Z., Zare, Z., Shakeri, H., Sabihi, S. S., & Esmaillzadeh, A. (2013). Effect of multispecies probiotic supplements on metabolic profiles, hs-CRP, and oxidative stress in patients with type 2 diabetes. *Annals of Nutrition & Metabolism*, *63*(1), 1–9.
3. Barengolts, E. (2016). Gut microbiota, prebiotics, probiotics, and synbiotics in management of obesity and prediabetes: review of randomized controlled trials. *Endocr Practi*, 22, 1224–1234.
4. Bermudez-Brito, M., Plaza-Díaz, J., Muñoz-Quezada, S., Gómez-Llorente, C., & Gil, A. (2012). Probiotic mechanisms of action. *Annals of nutrition & metabolism*, *61*(2), 160–174.
5. de Toro-Martín, J., Arsenault, B. J., Després, J. P., & Vohl, M. C. (2017). Precision Nutrition: A Review of Personalized Nutritional Approaches for the Prevention and Management of Metabolic Syndrome. *Nutrients*. *9*(8):913.
6. Festi, D., Schiumerini, R., Eusebi, L. H., Marasco, G., Taddia, M., & Colecchia, A. (2014). Gut microbiota and metabolic syndrome. *World journal of gastroenterology*, *20*(43), 16079–16094.
7. Fijan, S., Frauwallner, A., Langerholc, T., Krebs, B., Ter Haar Née Younes, J. A., Heschl, A., Mičetić Turk, D., & Rogelj, I. (2019). Efficacy of Using Probiotics with Antagonistic Activity against Pathogens of Wound Infections: An Integrative Review of Literature. *BioMed research international*, 7585486.
8. Huseini H.F., Rahimzadeh G., Fazeli M.R., Mehrazma M., and Salehi M. (2012). Evaluation of wound healing activities of kefir products. *Burns,* 38(5), 719–23.
9. Kadam, S., Shai, S., Shahane, A., & Kaushik, K. S. (2019). Recent Advances in Non-Conventional Antimicrobial Approaches for Chronic Wound Biofilms: Have We Found the 'Chink in the Armor'? *Biomedicines*, *7*(2), 35.
10. Kasińska, M. A., & Drzewoski, J. (2015). Effectiveness of probiotics in type 2 diabetes: a meta-analysis. *Polskie Archiwum Medycyny Wewnetrznej*, *125*(11), 803–813.
11. Kelly, C., Zheng, L., Campbell, E., Saeedi, B., Scholz, C., Bayless, A., Colgan, S. (2015). Crosstalk between microbiota-derived short-chain fatty acids and intestinal epithelial HIF augments tissue barrier function. *Cell Host & Microbe. 17*(5): 662-671.
12. Kesika, P., Sivamaruthi, B. S., & Chaiyasut, C. (2019). Do Probiotics Improve the Health Status of Individuals with Diabetes Mellitus? A Review on Outcomes of Clinical Trials. *BioMed research international*, *2019*, 1531567.

13. Kleerebezem, M., and Elaine E. Vaughan. (2009) "Probiotic and gut lactobacilli and bifidobacteria: molecular approaches to study diversity and activity." *Annual review of microbiology* 63: 269-290.
14. Lin, M. Y., & Yen, C. L. (1999). Antioxidative ability of lactic acid bacteria. *Journal of agricultural and food chemistry*, *47*(4), 1460–1466.
15. Lukic, J., Chen, V., Strahinic, I., Begovic, J., Lev-Tov, H., Davis, S. C., Tomic-Canic, M., & Pastar, I. (2017). Probiotics or pro-healers: the role of beneficial bacteria in tissue repair. *Wound repair and regeneration : official publication of the Wound Healing Society [and] the European Tissue Repair Society*, *25*(6), 912–922.
16. Mazloom, Z., Yousefinejad, A., & Dabbaghmanesh, M. H. (2013). Effect of probiotics on lipid profile, glycemic control, insulin action, oxidative stress, and inflammatory markers in patients with type 2 diabetes: a clinical trial. *Iranian journal of medical sciences*, *38*(1): 38–43.
17. Mihai, M. M., Preda, M., Lungu, I., Gestal, M. C., Popa, M. I., & Holban, A. M. (2018). Nanocoatings for Chronic Wound Repair-Modulation of Microbial Colonization and Biofilm Formation. *International journal of molecular sciences*. *19*(4):1179.
18. Mills, S., Stanton, C., Lane, J., Smith, G., & Ross, R. (2019). Precision nutrition and the Microbiome, Part I: Current state of the science. *Nutrients. 11*(4) : 923-968.
19. Ouwehand, Arthur C., Seppo Salminen, and Erika Isolauri, (2002). Probiotics: an overview of beneficial effects. In Lactic acid bacteria: genetics, metabolism and applications. *Springer, Dordrecht*, 279-289.
20. Rothschild, D., Weissbrod, O., Barkan, E., Kurilshikov, A., Korem, T., Zeevi, D., Segal, E. (2018). Environment dominates over host genetics in shaping human gut microbiota. *Nature. 555*(7695): 210-215.
21. Sikorska, H., & Smoragiewicz, W. (2013). Role of probiotics in the prevention and treatment of meticillin-resistant *Staphylococcus aureus* infections. *International journal of antimicrobial agents*, *42*(6), 475–481.
22. Sun, Z., Sun, X., Li, J., Li, Z., Hu, Q., Li, L., Hao, X., Song, M., & Li, C. (2020). Using probiotics for type 2 diabetes mellitus intervention: Advances, questions, and potential. *Critical reviews in food science and nutrition*, *60*(4), 670–683.
23. Valdéz J.C., Peral M.C., Rachid M., Santana M., and Perdigón G. (2005). Interference of *Lactobacillus plantarum* with *Pseudomonas aeruginosa in vitro* and in infected burns: the potential use of probiotics in wound treatment. *Clin Microbiol Infect*, 11(6), 472–479.
24. Zheng, P., Zeng, B., Liu, M., Chen, J., Pan, J., Han, Y., Xie, P. (2019). The gut microbiome from patients with schizophrenia modulates the glutamate-glutamine-GABA cycle and schizophrenia-relevant behaviors in mice. *Science Advances. 5*(2) : 8317-8326.